# nature research

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# **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all s	tatistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Со	nfirmed
	x	The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	×	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	×	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	x	A description of all covariates tested
	×	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	×	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	×	For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted Give $P$ values as exact values whenever suitable.
X		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	'	Our web collection on statistics for biologists contains articles on many of the points above

## Software and code

Policy information about <u>availability of computer code</u>

Data collection no software used

Data analysis

R (v. 3.6.1); R (v. 3.6.3) and R (v.4.0.2); R packages: [WGCNA (v. 1.69); topGO (v. 2.24.0); ComplexHeatmap (v. 2.1.0)] hisat2 (v. 2.1.0); htseq-count (v. 0.10.0); DESeq2 (v. 1.18.1); Cytoscape (v. 3.8.0); exonerate (v. 2.2.0); mafft (v. 7.397); dunn.test (v. 1.3.5); ggplot2 (v. 3.3.3); ggtern (v. 3.3.0)]; Metaboanalyst (v. 4.0); compositions (v. 2.0-1); vegan (v. 2.5.7); devtools (v.2.4.0); pairwiseAdonis (v.0.0.1), BLASTp (v.2.7.1+);  $\tau$ -coffee; iqtree2; iTOL (v.6);

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

#### Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-seq reads generated in this study have been deposited in Sequence Read Archive (BioProject ID: PRJNA685589 and BioSample accessions: SAMN17088123-SAMN17088147). The normalised expression data, differential expression results, ILP annotations and ILP sequences are available as supplementary data 3-6. Further data and scripts that support the findings of this study are available in Dryad with the identifier https://doi.org/10.5061/dryad.51c59zw7t. All other data are available from the corresponding author on reasonable request.

Field-spe	ecific reporting	
<u> </u>	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
Life sciences	Behavioural & social sciences	
	the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf	
Life scier	nces study design	
All studies must dis	close on these points even when the disclosure is negative.	
Sample size	Sample size was determined upon availability of termite individuals	
Data exclusions	No data were excluded.	
Replication	Exact procedures and data analysis steps are provided to ensure reproducibility of the study.	
Randomization	Termites were sampled at random among the available individuals of a given age or caste.	
Blinding	The fat body transcriptomic /lipidomic analyses and the lipidomic /metabolomic analyses on hemolymph occurred with relabeled samples ensuring blinding.	
We require information system or method list		
Eukaryotic  Palaeontol	cell lines Flow cytometry ogy and archaeology MRI-based neuroimaging	
	d other organisms	
Human research participants		
<b>▼</b> Clinical data		
Dual use research of concern		
Animals and	other organisms	
Policy information	about studies involving animals; ARRIVE guidelines recommended for reporting animal research	
Laboratory animals		

Wild animals

Macrotermes natalensis field colonies opened to provide animals in these experiments were followed over 20 years in Pretoria (South Africa) in an experimental field of the University of Pretoria (coordinates in Supplementary Table 8). Old female (small) workers (FW) and 20-years old queens (QT4) and kings (KT4) and male and female imagoes (QT0) were collected at swarming using nets and used to establish incipient colonies, all were sampled from the same colony (see Supplementary Table 1 for more details on sampling and replicates).

Field-collected samples

Hemolymph were collected from individuals cold-anesthetized in cryotubes, quickly frozen in liquid nitrogen and kept at -80°C until use. Then, termites were killed by decapitation and abdominal fat body was collected and loaded in a tube containing RNAlater buffer (Invitrogen) and kept at -80°C until use. For lipids and metabolites analyses, the fat body was nitrogen-frozen crushed in a tube which was immediately frozen in liquid nitrogen and kept in -80°C until use. For ploidy analyses, the fat body was collected from one individual and loaded in a tube containing 200  $\mu$ L of Cycletest PLUS DNA Reagent Kit buffer (Becton Dickinson) and kept in -80°C until use (see Supplementary Table 1 for more details on sampling and replicates).

Ethics oversight

Concerning insects no ethical approval was required. The study was conducted according to the Nagoya protocol. Samples (tissues and hemolymph) were exported at -80°C from Pretoria-South Africa to Bondy-France (permit 93010001).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Flow Cytometry

### **Plots**

Confirm that:
The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
All plots are contour plots with outliers or pseudocolor plots.
🗷 A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation	Fat bodies (see Supplementary Table 1 for more details on sampling and replicates) were processed by Flow Cytometric Analysis with a Cycletest PLUS DNA Reagent Kit using propidium iodide (BD Biosciences, Le pont de Claix) as described by manufacturer's instructions. All procedures were adapted from Nozaki & Matsuura.
Instrument	Accuri C6 Flow Cytometer (BD Biosciences, Le Pont de Claix, France). Stained nuclei were analyzed at an excitation wavelength of 488 nm and a detector equipped with an 585/45 bandpass filter.
Software	We analysed the data using CFlow Plus (BD Biosciences, Le Pont de Claix, France).
Cell population abundance	Approximately 1,000 cell nuclei were acquired for each measurement.
Gating strategy	Debris were removed on an FSC-A/SSC-A dotplot and doublet were eliminated with and PI-FL2-H/FL2-A dot plot. The nuclei were analyzed with a histogram PI-A. The 1C DNA peak was determined by the analysis of king's testis (sperm), allowing the identification of the 2C, 4C, and 8C peaks of the others samples.

**x** Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.